#19

Appl. No. 09/779,560
Atty. No. 58982.000002

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Marianne HARBOE

Serial Number: 09/779,560

Filed: February 9, 2001

Group Art Unit: 1652

Examiner: David J. STEADMAN, Ph.D.

For: METHOD OF PROVIDING POLYPEPTIDE PREPARATIONS WITH REDUCED ENZYMATIC SIDE ACTIVITIES

DECLARATION UNDER 37 C.F.R. § 1.132

Director of the United States Patent Office
Washington, D.C. 20231

Sir:

I, Peter Budtz, a citizen of the Kingdom of Denmark, residing at Hoffmeyersvej 21, DK 2000 Frederiksberg declare:

1) That I hold a degree as Master of Science in Chemical Engineering, and have worked in the field of enzyme application within the dairy and related industries for 18 years.

2) I am presently employed by Chr. Hansen A/S and hold the position of Senior Application Manager, Dairy Enzymes Technology.

3) That I have read and understand United States Patent Application No. 09/779,560 filed February 9, 2001.

4) That I understand that claims 1-32, have been rejected under 35 U.S.C. § 103(a) by the Examiner in United States Patent Application No. 09/779,560 as being obvious over Laustsen et al. (U.S. Patent 6,080,564) ("Laustsen"), in view of Larsen et al. (WO 95/29999) ("Larsen") and Heinsohn (U.S. Patent 5,215,908) ("Heinsohn") and/or further in view of Ward (Bioechnol 8:435-440, May 1990) ("Ward").

5) That I have read and understand Laustsen, Larsen, Heinsohn and Ward.

6) That Laustsen discusses the need and means for improving stability during the processing and storage of a particular lipase against degradation by proteolytic side activity. In particular Laustsen is directed toward the inactivation of Aspergillus protease, although amylase inactivation is discussed in examples 4 and 5. Further, in my opinion, the enzymes of Laustsen would not survive at a pH below 2.

7) That, while Laustsen discusses using a pH as low as 2.0, this pH is insufficient to achieve the goals of Application No. 09/779,560. For example, a pH of 2.0 will not inactivate glucoamylase. Further, Laustsen inactivates amylase, a different enzyme than glucoamylase, using at pH at either 3.5 or 10.7. Further, Laustsen does not mention the use of glucoamylase.

8) That the disclosure of Laustsen does not make it evident nor suggests to me that a pH lower than that disclosed in Laustsen would work to eliminate unwanted activity. On the contrary, using a pH below 2 in the context of treatment of enzymes is an uncommon practice, particularly for organic materials, such as proteins, sugars, and fats.

9) That Heinsohn relates to chromatographic purification of chymosin from unspecified enzymes and other impurities, and does not discuss glucoamylase or amylase activity. Further, the acid pre-treatment of pH 2-3 before chromatography according to example 1 is not sufficient to inactivate glucoamylase acitivity.

10) Further, examples 1 and 2 of the present application show that a pH below 2.0, e.g. pH 1.8, inactivates glucoamylase activity.

11) That neither Larsen, Heinsohn, nor Ward suggest using a pH below 2. In my opinion, the Applicant's use of a pH lower than 2.0 to inactivate at least one undesired enzymatic side activity is non-obvious in view of Laustsen, Larsen, Heinsohn and/or Ward.

The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signature: _____ Date: 30 sept. 2002

PATENT
Attorney Docket No. 58982.000002

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:	)	
	)	
Marianne HARBOE	)	Group Art Unit: 1652
	)	
Serial No: 09/779,560	)	Examiner: David J. STEADMAN, Ph.D.
	)	
Filed: February 9, 2001	)	

For: METHOD OF PROVIDING POLYPEPTIDE PREPARATIONS WITH REDUCED ENZYMATIC SIDE ACTIVITIES

DECLARATION UNDER 37 C.F.R. § 1.132

Under Secretary of Commerce for Intellectual Property
and Director of the United States Patent and Trademark Office
Washington, DC 20231

Sir:

I, Peter Budtz, state and declare as follows:

1. I have been awarded the degrees of Master of Science, chemical enginering from the Danish Technical University of Copenhagen.

2. I am an employee of Chr. Hansen A/S, and I presently hold the position of Senior Application Manager, Dairy Enzymes Technology.

3. I have 18 years of industry experience in the field of enzymology and application within the Dairy and related industries. That experience includes research and development in enzymes synthesis and utilization.

4. I have read and understand the above-identified patent application, including claims 1-32 thereof, as well as the Office Action issued by the U.S. Patent and Trademark Office on April 9, 2002. Appendix A includes claims 1-32 which I understand include some amendments made in response to the Office Action.

5. Based on my education and professional experience, I consider myself to be a person of ordinary skill in the art of this patent application.

6. I understand that claims 20, 21, 27, 29, 30 and 31 were rejected in the Office Action as indefinite because it "...is unclear from the specification and the claims as to the derivatives of a medium or an aspartic protease to which applicants refer...". Office Action, page 4. This assertion was based on the use of the word "derived" in those claims.

7. In my opinion, the use of the term "derived" does not introduce indefiniteness into these claims. Based on my experience, "derived" used in the context of the claims may mean obtained from a particular source (e.g., in claim 20 from the cultivation of a microorganism which, during cultivation, produces an aspartic protease and at least one undesired enzymatic activity). I can readily ascertain the utilization of and the scope of each of the above claims which use the term "derived".

8. I understand that one of the rejections in the Office Action is that of claim 32 as containing subject matter which was not described in the specification in such a way as to reasonably convey to a person skilled in the relevant art that the inventor, at the time the application was filed, had possession of the invention recited in that claim.

9. I understand that a specification of a patent application is deemed to describe the entire subject matter of a particular claim under consideration if the specification conveys, with reasonable clarity to a person of ordinary skill in the art, that the inventor was in possession of the invention recited in the claim of interest. It is also my understanding that, to satisfy that requirement, an Applicant does not have to utilize any particular form of disclosure to describe the subject matter of the claim under consideration. For instance, I understand that the necessary disclosure may be included in working examples or in a more general description of the invention (or a combination thereof).

10. I understand that claim 32 is directed to a method of making a polypeptide preparation having a defined content of undesired enzymatic side activities, i.e., at such a level that such activities do not restrict the applicability of the polypeptide preparation for its intended use. The method comprises the following steps:

(i) providing a medium having a pH of 2.0 or higher that includes at least one desired polypeptide having an enzymatic activity, and at least one undesired enzymatic side activity, and

(ii) subjecting the medium to a pH of less than 2.0 for such a period of time which is sufficient to at least partially inactivate at least one of such undesired enzymatic side activities.

The polypeptide has an aspartic protease activity and it can be an animal aspartic protease, a plant aspartic protease or a microbial aspartic protease. Further, the aspartic protease is derived from a naturally-produced aspartic protease by adding or deleting one or more amino acids or substituting one or more amino acids.

11. As I understand it, this claim was rejected (as summarized above) because it was stated in the Office Action that the specification teaches two representative species of the aspartic protease, i.e., an amylase or glucoamylase-prochymosin fusion protein, and, allegedly, the specification does not describe any other representative species by any identifying characteristics or properties other than the functionality, i.e., of being an aspartic protease derived from a naturally-produced aspartic protease (as described above, i.e., by addition, deletion, or substitution of one or more amino acids).

12. In my opinion, the specification provides a teaching which is commensurate in scope with that of claim 32. There are many passages in the

application which describe aspartic protease made by or derived from various organisms. For example, at page 8, lines 3-5, the inventor states that non-recombinant and recombinant microorganisms which are useful in the production of aspartic protease include bacterial species and yeast species, "...such as those mentioned above". The yeast species "mentioned above" include "...fungal species *Rhizomucor miehei* and *Rhizomucor pusillus* and protease naturally-produced by the fungal species *Cryphonectria parasitica*...[and] other fungal species including *Rhizopus* species, *Physarum* species and *Pencillium* species, and *Bacillus* species." See page 2, lines 27-32. This description of fungi is supplemented by the following disclosure of filamenteous fungal species which can be used to make aspartic proteases: "...species of *Aspergillus*, e.g., *Aspergillus oryzae*, *Aspergillus nidulans* or *Aspergillus niger* including *Aspergillus nigervar. awamori*.[...] a *Fusarium* species, e.g., *Fusarium oxysporum* or of a *Rhizomucor* species such as *Rhizomucor miehei* or a *Trichoderma* species including *Trichoderma reseei* and strains of *Cryphonectria* species including *Cryphonectria parasitica*..." See page 8, lines 9-14.

13. Additional examples of organisms which can produce aspartic protease are described as follows:

> In accordance with the invention, the desired polypeptide in the preparation that is obtained is an aspartic protease derived from the group consisting of an animal aspartic protease including a mammalian aspartic protease including pro-chymosin, chymosin, pepsinogen and pepsin, a plant aspartic protease and a microbial aspartic protease. A mammalian aspartic protease can be derived from any mammal species such as a ruminant species including a bovine species, an ovine species, a caprine species, a deer species, a buffalo species, an antelope species and a giraffe species, a *Camelidae* species including *Camelus dromedarius*, a porcine species, an *Equidae* species and a primate species. As it is mentioned above, a commonly used milk clotting enzyme preparation is based on extracts

> of stomach tissues of mammals naturally producing a milk clotting aspartic protease. Accordingly, the method of the invention is applicable to such preparations, including preparations of aspartic proteases derived from a naturally producing aspartic protease by the addition or deletion of one or more amino acids or substituting one or more amino acids herein.

Page 8, lines 22-35. Additional disclosure of various species is included in the working examples (such as, Examples 1 and 2).

14. At least the aforementioned passages of the specification convey to me that Applicant was in possession of the invention of the entire scope of claim 32.

15. I also note the rejection in the Office Action of claims 1-32 for a lack of enablement of the full scope of those claims. As I understand it, claims 1-32 were rejected because, according to the text of the Office Action, the specification enables a method of preparing chymosin with reduced activities of glucoamylase, peptidase, amylase, cellulase, phosphatase, and protease by treating a non-acidophilic cell medium, including such enzymes, at a pH of 1.6 to 1.8 for a time sufficient to reduce the undesired activities of the aforementioned enzymes (as compared to the untreated, respective enzymes). Office Action, pages 5-6. Nonetheless, according to the Office Action, as I understand it, it was held that the specification does not provide enablement for a method of producing any desired polypeptide having a reduced content of any enzymatic side activities by treating any medium with any pH of less than 2, as it was alleged is covered by the claims.

16. It was explained to me that a specification of a patent application satisfies the enablement requirement if the scope of the claims in question is enabled, so that a person of ordinary skill in the art would be able to make and use the claimed invention without undue experimentation. I understand that the question of undue

experimentation is a matter of degree. For example, if some experimentation is necessary, it does not preclude enablement, so long as the experimentation is not unduly extensive. I also understand that several factors need to be considered in determining if undue experimentation is required. Such factors are summarized in the Office Action and repeated below:

(1) the quantity of experimentation necessary,

(2) the amount of direction or guidance presented,

(3) the presence or absence of working examples,

(4) the nature of the invention,

(5) the state of the prior art,

(6) the relative skill of those ordinarily skilled in the art,

(7) the predictability or unpredictability of the art, and

(8) the breadth of the claim(s).

Office Action, page 5.

17. Based on the above-summarized factors for the "enablement" requirement, it is my opinion that the specification provides a substantially broader scope of enablement than is stated in the Office Action (i.e., preparation of chymosin with reduced undesired activities of glucoamylase, peptidase, amylase, cellulase, phosphatase, and protease by treating a non-acidophilic cell medium including such enzymes at a pH of 1.6 to 1.8 for a sufficient time to reduce the undesired activities). It is further my opinion that the specification provides enablement for the full scope of claims 1-32.

18. I agree with the text of the Office Action that examples 1 and 2 provide at least the scope of enablement which was alleged in the Office Action on pages 5 and 6

(summarized above). My review of the specification indicates that the scop of enablement is broader than stated in the Office Action. For instance, the pH values specifically tested in Example 1 include pH of 1.15, 1.30, 1.48 and 1.61, in Example 2, pH of 1.6, 1.7 and 1.8, and in Example 3, pH of 1.7. The specification also discloses that the term "polypeptide" includes peptides of two or more amino acids, such as peptides, oligopetides or proteins (page 4, lines 31-35), and raw materials and intermediate products used to manufacture final products containing the polypeptides (page 5, lines 9-14). Examples of such polypeptides are preparations of enzymes made by extraction from tissues of higher organisms or made by cultivation of microorganisms, e.g., milk clotting enzymes of animal and microbial origin using either organisms producing such an enzyme or using recombinant host microorganisms having an inserted gene expressing the milk clotting enzyme (page 5, lines 14-20).

The inventor also teaches that any starting or intermediate materials used to make a preparation containing a desired polypeptide or the final product can be used in the method of the invention. She exemplifies such materials as comprising media derived from the cultivation of microorganisms that, in the course of cultivation, produce one or more desired polypeptide and at least one undesired enzymatic side activity (page 6, lines 30-35). A specific example of such media are identified as those derived from the cultivation of animal cells, plant cells and microbial cells, "...including cells of a bacterial species such as a gram negative bacterial species including *E. coli* and a gram positive species including a *Bacillus species*, a yeast species and a species of filamentous fungi" (page 7, lines 2-4). Specific examples of such media are those derived from the cultivation of cells of various yeast species, such as *Saccharomyces cerevisiae*, a methylotrophic yeast species, such as *Pichia pastoris* and a

Klyuveromyces species, and media derived from cultivation of species of filamentous fungi, such as *Aspergillus* species, *Cryphonectria* species, *Fusarium* species, *Rhizomucor* species and *Trichoderma* species (page 7, lines 6-9).

The specification also provides a disclosure of what constitutes "undesired enzymatic side activity". Such activity is identified as "any enzymatic activity, the presence of which in a polypeptide preparation is undesired for any reason such as detrimental or toxic effects occurring upon application or administration of the polypeptide preparation" (page 5, lines 24-27). Such undesired enzymatic side activities are exemplified by degradation of valuable components in a food product and immunologically adverse effects occurring when a pharmaceutically active polypeptide is administered, protease activity, starch degrading activity, peptidase activity, lipase activity, cellulase activity, lactase activity, hemicellulase activity, glucoamylase activity and phosphatase activity (page 5, lines 27-32).

As discussed above, the specification also provides a disclosure of how to make many other species of polypeptides with enzymatic activities, such as aspartic protease which may be an animal aspartic protease, including a mammalian aspartic protease, a plant aspartic protease and a microbial aspartic protease. Details of such proteases are discussed in paragraph 10, above. Example 3 provides a specific disclosure of how to make the claimed polypeptide from certain microbial and animal rennet products, i.e., Hannilase™ 195, a microbial coagulant produced by *Rhizomucor miehei*, Hannilase™ 2100, also a microbial rennet produced by *Rhizomucor miehei*, CHY-MAX™, a bovine chymosin produced by *Aspergillus niger var. awamori*, Modilase™ 195, an oxidised, thermolabile coagulant derived from *Rhizomucor miehei* and Thermolase™, a microbial coagulant produced by *Cryphonectria-parasitica*.

19. Examples 1-3 and general disclosure of the specification teaching the applicability of the method of the described invention to other polypeptides with an enzymatic activity and other undesired enzymatic activities (such as starch degrading activity, peptidase activity, lipase activity, cellulase activity, lactase activity, hemicellulase activity, glucoamylase activity and phosphatase activity) (e.g., page 5, lines 31-32), enable me to conduct the method of claim 1 for its full scope. The presence of the working examples in conjunction with a more generic disclosure in the remainder of the application instructing how to carry out the claimed method for the described preparations having described enzymatic activity and undesired enzymatic side activity provides such enablement. In my opinion, if any experimentation was necessary, only a reasonable amount of experimentation would be needed to operate the method for preparations with many different polypeptides covered by claim 1 having an enzymatic activity to reduce a variety of undesired side activities as covered by claim 1. The nature of the invention is such that it would be relatively easy for me to conduct the method for the full scope of claim 1 based on the quantity and quality of the disclosure in the specification. While the art may have some unpredictability, in my opinion, working Examples 1-3, together with the remainder of the specification (as summarized above), decrease the level of unpredictability to a reasonable one because the particular species of polypeptides having an undesired enzymatic activity discussed in the application make it apparent that other polypeptides with other undesired enzymatic activities encompassed by claim 1 could be used in the method of claim 1 in a similar manner.

20. Since claims 2-32 depend from claim 1, I understand that th y have a narrower scope than claim 1. Since claim 1 is fully enabled, claims 2-32, in my opinion, are also fully enabled by the specification.

The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

Date: Copenhagen 30. September 2002 By:

Peter Budtz